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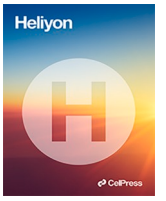

Research article

Spatiotemporal variations in the occurrence of *Campylobacter* species in the Bloukrans and Swartkops rivers, Eastern Cape, South Africa

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ABSTRACT

An increase in the incidence of *Campylobacter* species in rivers raises concerns on the safety of river water for humans who get exposed to river water. This study examines the spatiotemporal dynamics of *Campylobacter* species in the Bloukrans and Swartkops rivers, analysing patterns of its occurrence in relation to meteorological conditions, physicochemical parameters, seasons, and sampling sites. Physico-chemical parameters and meteorological conditions were measured during water sampling from various sites along the rivers over a year, while Polymerase Chain Reaction (PCR) was utilised to detect *Campylobacter* genus-specific genes and selected antibiotic-resistant genes. *Campylobacter* was detected in 66.67% (Bloukrans River) and 58.33% (Swartkops River). In the Bloukrans River, multi-drug resistance genes *cmeA* (20%), *cmeB* (65%), *cmeC* (10%), were detected while and *tetO* was detected at 70%. In the Swartkops River, the corresponding prevalence were 28%, 66.67%, 28.56%, and 76%. The study indicates that sampling season did not significantly impact *Campylobacter* prevalence. However, variation in *Campylobacter* occurrence exists among different sites along the rivers, reflecting the influence of site proximity to potential contamination sources. The study suggests that *Campylobacter* infection may be endemic in South Africa, with rivers serving as potential sources of exposure to humans, thereby contributing to the epidemiology of campylobacteriosis.

1. Introduction

Campylobacter species are etiological agents for gastrointestinal and extra-gastrointestinal infections in humans [1,2]. Although most *Campylobacter* infections are linked to the consumption of contaminated food, human may also be infected through contaminated water. Faecal contamination of surface water from different anthropogenic sources is a major contributor of *Campylobacter* in rivers and *Campylobacter* from water sources has been linked to waterborne outbreaks [3–6]. The emergence of *Campylobacter* species in water sources therefore presents a public health risk for humans who may be exposed to river water.

Previous studies suggest that *Campylobacter* occurrence in rivers is influenced by seasons and anthropogenic activities in the watersheds draining the rivers. Seasonal variation in levels of *Campylobacter* contamination in rivers has been reported globally, however few studies in Africa have investigated the influence of seasonal variation on the occurrence of *Campylobacter* in rivers [7–11]. Seasonal variations can affect the occurrence, and recovery of *Campylobacter* from water, as well as the period of higher risk of

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exposure to the water users. In addition to seasonal variations, the occurrence of *Campylobacters* species in rivers is also influenced by anthropogenic activities in the catchment [1,7]. *Campylobacter* in river water mainly originates from anthropogenic activities which include faecal matter from animals, agricultural runoff, and wastewater treatment plant effluent [12,13].

Bloukrans and Swartkops rivers lie in the Eastern Cape Province of South Africa. Both rivers are important for recreational, and agricultural purposes for the locals. Yet, these rivers are at risk of *Campylobacter* pollution. These rivers are affected by faecal pollution from livestock production , dilapidated wastewater treatment plants, and agricultural practices. Furthermore, high population, unplanned settlements, poor management of water resources, and wastewater infrastructure failure are leading to deteriorating water quality in these freshwater bodies. In addition to microbial pollutants from the community, antibiotic residues from healthcare facilities and wastewater also end up in the rivers [14,15,16].

The antibiotic residues that end up in rivers act as a selection pressure for the antibiotic-resistant microbial pathogens in the environment. Resistance of pathogens to antibiotics that are used for therapeutic purposes in human medicine is a major public health concern because the infections caused by these microorganisms are becoming more difficult to treat. This phenomenon leads to an increased duration of hospitalisation, high morbidity, and mortality [17]. It is crucial to investigate the distribution of *Campylobacter* species across different locations in rivers in different seasons of the year to identify potential sources of contamination, estimate the risk of waterborne infections, and formulate targeted strategies to minimise the impact on human health. The paucity of comprehensive understanding of these variations will hinder the implementation of effectual preventive measures and prompt responses to emerging public health threats associated with *Campylobacter* infections in the Bloukrans and Swartkops river systems. This study investigates the spatiotemporal patterns of *Campylobacter* species occurrence in Bloukrans and Swartkops rivers, examining influences from seasonality, and proximity to potential contamination sources. Variations in the frequency of *Campylobacter* occurrence in specific sites/location along the rivers and periods is expected, indicating a fluctuating risk of waterborne infections. This study investigates the spatiotemporal dynamics of *Campylobacter* species in river water considering factors such as meteorological conditions, physicochemical parameters, season of the year, and sampling site. The innovation of this study lies in its pioneering nature, being the first to employ non-culture based method to investigate spatiotemporal variations in the occurrence of *Campylobacter* species in the Bloukrans and Swartkops rivers of the Eastern Cape, South Africa. Furthermore, this is the first study explores the complex interactions between meteorological conditions, physicochemical characteristics and dynamics of waterborne pathogens in this specific geographical region.

2. Materials and methods

2.1. Study area

This study was conducted was in the Bloukrans and Swartkops river catchments in the Eastern Cape, South Africa. Water sampling was conducted at different locations along the selected rivers. The Bloukrans River flows through the small town Makhanda, Eastern Cape, South Africa. Despite poor quality and low quantities of water, the Bloukrans River supports communities around it, and the state of this river may have adverse effects on public health. The Swartkops River flows through Uitenhage, Kwanobuhle, Despatch,

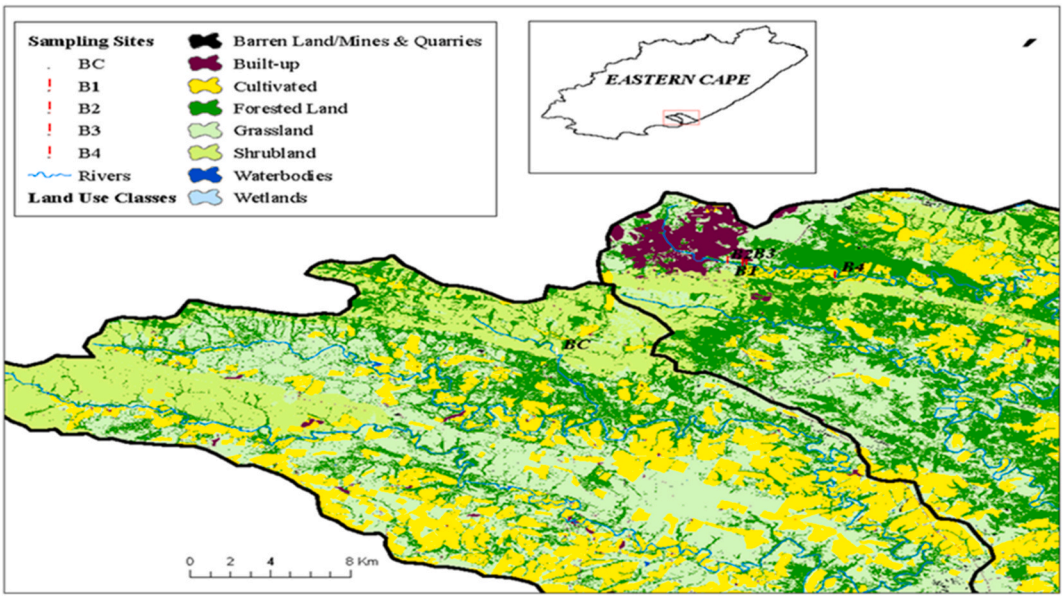

Fig. 1. Land-use within the catchment and location of the sampling sites (sites BC, B1, B2, B3, and B4) on the Bloukrans River, Eastern Cape, South Africa.

Motherwell, Zwide, and Blue Water Bay in the Nelson Mandela Bay Metro Municipality, Eastern Cape, South Africa.

A total of 11 sampling sites were selected on the two rivers (i.e. five and six sampling sites from Bloukrans and Swartkops Rivers, respectively) from the upper and middle reaches of the rivers. The sampling points are spatially distributed along the rivers and were classified according to the predominant anthropogenic activities in the area around the sampling site. On the Bloukrans River, five (5) sites were selected. One site (BC) is a control site (33°22'08.4″S 26°28'30.0″E) and this site is least impacted by human activities except for traditional/cultural rituals that take place. Site B1 (33°18'51.4″S 26°33'06.0" E) is influenced by stormwater, and human settlements and is a place where animals (livestock) graze and drink water while another site (B2, 33°18'56.2" S 26°33'30.8″E) is influenced by wastewater effluent. The fourth site (B3, 33°18'55.5″S 26°33'36.5" E) lies in an area where the predominant activity is agriculture while the last site (B4, 33°19'24.0″S 26°36'00.4" E) is a point of human exposure (recreation and cultural activities) and is influenced by upstream activities (Fig. 1). For the Swartkops Rivers, the control site (SC) is relatively pristine and least impacted by anthropogenic activities (33°44'10.7″S, 25°19'11.0" E). Site S1 (33°45'06.9″S, 25°20'34.6″E) lies within an agriculture settlement area immediately downstream of the control site. The third site (S2) lies in an industrial area (33°47'31.8″S 25°24'28.3" E) while the fourth site (S3) is immediately after a wastewater discharge point (33°47'11.66″S, 25°26'00.46″E). The fifth site (S4, 33°47'31.5″S 25°27'51.5" E) lies after Wastewater Treatment Plant (WWTP) discharge point and in an agricultural area while the last site (S5,33°48'40.2" S 25°31'49.1″E) is in an area influenced by stormwater and industrial waste, human settlements, and animals (Fig. 2).

Before sampling, a sanitary inspection was conducted at each site. This inspection is a physical survey that identifies the sources and potential sources of microbiological pollution for each site. Identifiable sources of pollution (point sources) such as WWTPs were noted. Diffuse sources (non-point sources) such as agricultural runoff, urban runoff, faecal matter from livestock, and solid waste dumpsites were also identified. The natural and anthropogenic activities at the sampling point, downstream and upstream of the sampling point, and within the catchment were also identified.

2.2. Sample collection

Water samples from the sampling points were collected from June 2021 to April 2022. The sampling period considered all the seasons of the year (autumn, spring, summer and winter). This study used the seasonal boundaries as reported in the study by Kruger and Nxumalo [18]. In South Africa, summer starts in December and end in February while autumn begins in March and ends in May. This followed by winter which begins in June and lasts till August while spring is in September and November [18].

For Bloukrans River, water sampling was conducted on the 17th and 18th of June 2021 (winter), 25th and 26th of October 2021 (spring), 7th, 23rd, and 24th of February 2022 (summer); and 5th, 20th and 21st April 2022 (autumn). For Swartkops River, water samples were collected on 14th and June 15, 2021 (winter), 25th and October 26, 2021 (spring), 3rd, 4^{th,} and February 28, 2022 (summer); 4th, 22nd, and April 23, 2022 (autumn). The total of water samples collected from the selected sampling sites was 30 and 36 samples for the Bloukrans, and Swartkops rivers, respectively.

Selected meteorological conditions (temperature, humidity, atmospheric pressure, and precipitation) for each day of sampling were obtained from the South African Weather Services (SAWS). At each sampling site, the volume of water samples collected was 2 L. Onsite analysis of physicochemical parameters was conducted for dissolved oxygen (mg/L), temperature (°C), electrical conductivity

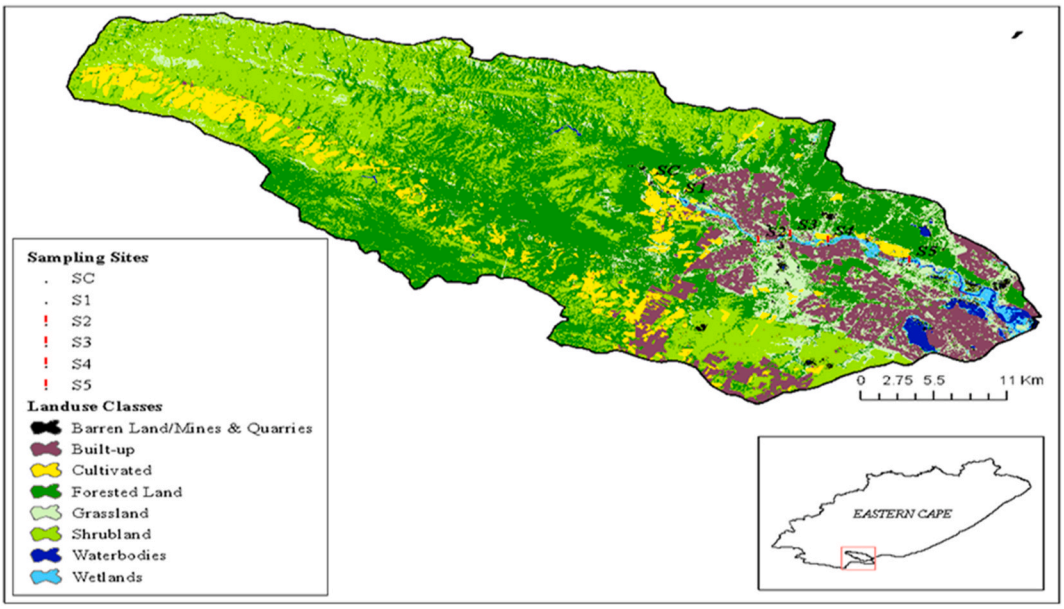

Fig. 2. Land-use within the catchment and location of the sampling sites (sites SC, S1, S2, S3, S4, and S5) on the Swartkops River (right), Eastern Cape, South Africa.

parameter (μs/cm), pH (Hanna Instruments, South Africa) and turbidity in Nephelometric Turbidity Units (NTU) (Turbidimeter, Eutech Instruments, USA). Physicochemical analysis was conducted using methods prescribed by the American Public Health Association (APHA). Before measuring the parameters, the instruments were calibrated based on the manufacturer's instructions. Measurements were obtained by submerging the probe in the river water and steadily holding it to obtain precise readings. De-ionised water was used to rinse the probes to avoid cross-contamination [19]. The water samples were preserved at low temperatures during transportation to the laboratory and preserved at 4 °C.

2.3. Sample processing

Sample processing involved centrifuging 2 L of water at a maximum of 14000 g for 60 min (Avanti® J-E Centrifuge Beckman Coulter, Inc. USA). Afterwards, the supernatant was then transferred into a steriled container and filtered using sterile 47 mm/0.45 μm cellulose nitrate membrane filters (Whatman, plc, UK), via a membrane filter assembly. The membrane filters were placed in sterile Eppendorf tubes. The pellets from the centrifuge tubes were scrapped off and added to sterile Eppendorf tubes containing the membrane filters and ready for DNA extraction. The water samples were centrifuged with the supernatant filtered because the river water samples contain bacterial cells as well as solid particles that may have bacterial cells trapped or attached to them in the river. Therefore, through centrifugation, the free bacterial cells would be pelleted alongside with those trapped in the solid particles. However, some bacteria might remain in the liquid phase of the sample, which could also be collected as a residue on the filter membrane using filtration. To capture cells that may have remained in the supernatant, the supernatant was filtered, and cells were collected as a residue on a filter membrane. *Campylobacter* spp. occur in very low numbers in environmental waters, relative to background flora. Therefore, by pooling cells in the pellets and residue, the number of recoverable cells is increased.

2.4. DNA extraction

The collected pellets and filters were subjected to DNA extraction. A genomic DNA isolation kit (Qiagen Kit, DNeasy PowerSoil Pro Kit, Thermo Fisher Scientific, Germany) was used to extract DNA based on the protocol provided by the manufacturer. Furthermore, a [20] UV–Vis Spectrophotometer (ThermoFisher Scientific, Lenexa KS, USA) was used to ascertain the quality and concentration of the extracted DNA. DNA samples that recorded a 260/280 ratio in the range of 1.69–2.2 were considered good quality and used for Polymerase Chain Reaction (PCR).

2.5. Detection of Campylobacter and ARGs using standard Polymerase Chain Reaction (PCR)

The primers C412F and C1228R were used to detect the *Campylobacter* 16S rRNA [21]. Detection of hippuricase-positive *Campylobacter* was done by targeting the *hipO* gene [22]. Tetracycline-resistant genes were detected using the *tetO* primers while the primers *cmeA, cmeB, and cmeC* were used to detect, *Campylobacter* multi-drug resistant genes. The primers used for *cmeA*, *cmeB*, *cmeC*, and *tetO* are specific for *Campylobacter jejuni* [23,24]. Resistance to erythromycin resistance was determined by detecting the A2074G and A2075G point mutations of *Campylobacter* on the 23s rRNA gene. The primers used in this study were manufactured by Inqaba Biotech, South Africa. The PCR was carried out using the primer sets and optimised protocols as described in Tables 1 and 2 respectively. The PCR reaction mixture was 50 μL and consisted of the following; 25 μL EmeraldAmp GT PCR master mix (Takara Bio

Table 1
Primers used to detect *Campylobacter* and antibiotic-resistant genes.

Name	Primers (5'-3')	Amplicon size (bp)	References
C412F/C1228R	F-GGATGACACTTTTCGGAGC R- CATTGTAGCACGTGTGTC	816	Linton, Owen and Stanley [25]
hipO	F-GAAGAGGGTTTGGGTGGT R-AGCTAGCTTCGCATAATAACTTG	735	On and Jordan [22]
cmeA	*cmeA*	435	De Vries et al. [24]
cmeB	F-TAGCGGCGTAATAGTAAATAAAC	444	
cmeC	R- ATAAAGAAATCTGCGTAAATAGGA	431	
	cmeB		
	F-AGGCGGTTTTGAAATGTATGTT		
	R-TGTGCCGCTGGGAAAAG		
	cmeC		
	F-CAAGTTGGCGCTGTAGGTGAA		
	R-CCCCAATGAAAAATAGGCAGAGTA		
tetO	F-GGCGTTTTGTTTATGTGCG	559	Gibreel et al. [23]
	R-ATGGACAACCCGACAGAAGC		
23S rRNA at position 2074/2075	23S rRNA at position 2074	485	Alonso et al. [26]
	F-TTAGCTAATGTTGCCCGTACCG	485	
	R-AGTAAAGGTCCACGGGGTCTCG		
	23S rRNA at position 2075		
	F-TTAGCTAATGTTGCCCGTACCG		
	R-TAGTAAAGGTCCACGGGGTCGC		

Inc, China), 2 μL forward, 2 μL reverse primers, 2 μL template DNA, and 19 μL molecular grade water. The amplicons were visualised by electrophoresis on 1.5% agarose gels (CSL-AG100, Cleaver Scientific Ltd. Warwickshire, UK) and the gel images were captured using a UV machine (molecular imager ChemiDocTM XRS+, BIO-RAD).

2.6. Statistical analysis

To investigate the temporal dynamics, logistic regression was performed to investigate the relationship between the meteorological conditions of the sampling days (independent variables) and the detection of *Campylobacter* (dependable variable). Additionally, logistic regression was conducted to determine the relationship between physicochemical parameters, the season of the year, sampling site (independent variables), and *Campylobacter* detection (dependable variable). The statistical analyses were conducted using R software version 4.2.0. A negative coefficient (b) indicated that an increase in the independent variable is associated with a decrease in the detection of *Campylobacter*. A positive "b" means that an increase in the independent variable is associated with an increase in the detection of *Campylobacter*. The independent variable Odds Ratio (OR) represents the factor by which the odds change for the dependent variable given a unit increase in the independent variable (values > 1 indicate an increase in odds of *Campylobacter* occurrence while values < 1 indicate a decrease in the odds of *Campylobacter* detection). *P*-values ≤ 0.05 were regarded as significant.

3. Results

3.1. Physico-chemical characteristics of the river water

Results for physico-chemical analysis of the river water showed that the highest river water temperature was 24.98 °C in summer while the lowest was 13.1 °C for the Bloukrans River. The mean water temperature across different sites along the river was 18 °C to 20.1 °C while dissolved oxygen (DO) concentration ranged from 2.17 to 9.05 mg/l (Fig. 3). Dissolved oxygen was highest (>8–9.6 mg/l) for the control site (BC) throughout all the seasons and lowest at site B1 (≤5.0 mg/l). The sites (B1, B2, and B3) that recorded low dissolved oxygen concentration also recorded high electrical conductivity (>1000 μs/cm), and high turbidity. The mean pH ranged from 7.3 to 7.5 as shown in Fig. 3.

For the Swartkops River, the mean pH ranged from 7.1 to 7.62 (Fig. 3). The lowest temperature was 15.3 °C in winter while the highest was in summer (26.12 °C). The mean temperatures across the different sites ranged from 19.9 to 21.6 °C. Similar to results obtained from the Bloukrans River, the control sites recorded high dissolved oxygen (>8 mg/l) while human-impacted sites recorded lower dissolved oxygen (<4 mg/l). The electrical conductivity and turbidity were high (>1000 μs/cm and 40 NTU respectively) for the sites impacted by anthropogenic activities similar to what was observed in the Bloukrans River. Fig. 3 shows the mean physicochemical parameters across the sampling sites for the Swartkops River.

3.2. Prevalence of Campylobacter and selected antibiotic resistant genes

In this study, the prevalence of *Campylobacter* was 66.67 % (20/30) for the Bloukrans River, and 55.33% (21/36) for the Swartkops River. Sequence analysis of the PCR products obtained was done by BLAST. All of the products correspond to *C. jejuni,* and this was confirmed based on percent identity (96%). Additionally, hippuricase-positive *Campylobacter* were detected in 46.7% (14/30) of the Bloukrans River and 44.4% (16/36) of the Swartkops River.

Furthermore, *cmeA, cmeB* and *cmeC* genes were detected at 20%, 65% and 10% respectively in the 20 Bloukrans River water samples which were positive for *Campylobacter*. For the Swartkops River water samples, the prevalence of *cmeA, cmeB* and *cmeC* was 28%, 66.67% and 28.56%. Tetracycline resistance genes were detected in 70% (Bloukrans River) and 76% (Swartkops River). For both rivers, A2074G and A2075G point mutations on 23S rRNA were not detected. A2074G and A2075G point mutations of *Campylobacter* on the 23S rRNA gene are linked to erythromycin resistance.

Table 2
Primers and the PCR amplification conditions for the genes detected in the study.

Primer/Target gene	Initial Denaturation		Cycles	Denaturation		Annealing		Elongation		Final elongation		Reference
	°C	Time		°C	Time	°C	Time	°C	Time	°C	Time	
C412F/C1228R	94	1 min	35	94	1 min	58	1 min	72	4 min	72	5 min	Linton, Owen and Stanley [25]
hipO	94	1 min	35	94	1 min	58	1 min	72	4 min	72	5 min	On and Jordan [22]
cmeA, *cmeB*,	94	7 min	30	94	1 min	50	1.5 min	72	3 min	72	5 min	De Vries et al. [24]
cmeC	94	7 min	30	94	1 min	52	1.5 min	72	3 min	72	5 min	De Vries et al. [24]
tetO	95	1 min	35	95	15s	52	1 min	72	1 min	72	4 min	Gibreel et al. [23]
23S rRNA (at position 2074/2075)	95	5 min	35	95	1 min	59	30s	72	30s	72	4 min	Alonso et al. [26]

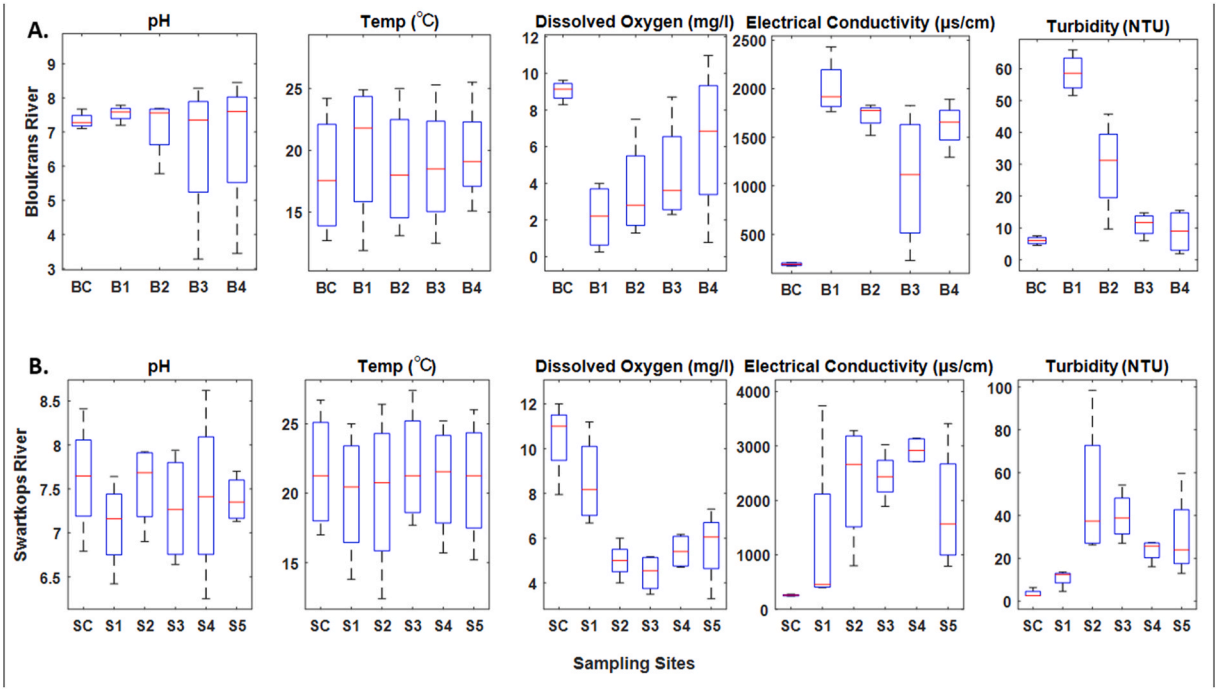

Fig. 3. Spatial variations for physiochemical characteristics (pH, Temperature, Dissolved Oxygen, Electrical conductivity and Turbidity). Panel A shows the results for water samples collected from the Bloukrans River, Eastern Cape, South Africa while panel B shows the results for water samples collected from the Swartkops River, Eastern Cape, South Africa.

3.3. Influence of river water physico-chemical characteristics on Campylobacter detection

Logistic regression analysis shows that generally, physicochemical parameters did not have a significant relationship with the detection of *Campylobacter* occurrence ($\chi 2 = 25.9$, $p = 0.001$, $n = 20$) ($\chi 2 = 13.41$, $p = 0.02$, $n = 24$) for both the Bloukrans and Swartkops rivers, respectively. For the Bloukrans River, an increase in pH had a negative effect on the detection of *Campylobacter* ($b = -54.5$, $OR = 0$) but the relationship is not significant ($p = 0.999$). The temperature also had a negative effect on the detection of *Campylobacter* ($b = -15.87$, $OR = 0$) but the relationship is not statistically significant ($p = 0.998$). Similarly, dissolved oxygen had a negative effect on the detection of *Campylobacter* although the relationship is not significantly significant ($p = 0.998$, $OR = 0$, $b = -22.78$). In contrast, electrical conductivity had a positive effect on the detection of *Campylobacter* but the relationship is not statistically significant ($p = 0.998$, $OR = 1.15$, $b = 0.14$). The relationship between turbidity and the detection of *Campylobacter* was not statistically significant ($p = 0.998$, $OR = 0.02$, $b = -3.94$). For the Swartkops River, an increase in pH has a negative influence on the detection of *Campylobacter* ($b = -0.09$, $OR = 0.92$) but the relationship is not significant ($p = 0.935$). The results showed that temperature also had a positive influence on the detection of *Campylobacter* ($b = 0.07$, $OR = 1.08$) but the relationship is not statistically significant ($p = 0.699$). Similarly, dissolved oxygen had a negative influence on the detection of *Campylobacter* although the relationship is not significantly significant ($p = 0.183$, $OR = 0.37$, $b = -1$). Contrary, electrical conductivity and turbidity both had a positive influence on the occurrence of *Campylobacter* ($b = 0$ for both) but this relationship was not significant ($p = 0.829$, $OR = 1$ for electrical conductivity, and $p = 0.973$; $OR = 1$ for turbidity).

3.4. Spatial occurrence of Campylobacter species in river water

Overall, the sampling site had a significant effect on the detection of *Campylobacter* for both the Bloukrans ($\chi 2 = 12.31$, $p = 0.015$) and Swartkops ($\chi 2 = 19.89$, $p = 0.001$) rivers. Variation in *Campylobacter* prevalence is observed amongst the different sites for both the Bloukrans and Swartkops rivers. *Campylobacter* was detected at Sites B1, B2, B3, and B4, and Sites S2, S3, S4, and S5, of Bloukrans and Swartkops respectively. In contrast, there was no detection of *Campylobacter* at the control sites for both the Bloukrans and Swartkops rivers, BC and SC, respectively. *Campylobacter* was also not detected at the site (S1) that is immediately downstream of the control site for the Swartkops River.

3.5. Temporal occurrence of Campylobacter in the rivers

Meteorological conditions on the sampling days were recorded for both the Bloukrans and Swartkops rivers. The mean values for meteorological conditions on the sampling days for each season are presented in Table 3. Statistical analysis conducted to investigate

the influence of meteorological conditions (air temperature, humidity, pressure, and precipitation) on the detection of *Campylobacter*, show that these conditions had no significant effect on the detection of *Campylobacter* for Bloukrans ($\chi2 = 2.62$, $p = 0.624$, $n = 20$) and Swartkops rivers ($\chi2 = 3.63$, $p = 0.458$, $n = 24$).

In terms of temporal occurrence, *Campylobacter* was detected in water samples from both rivers and for all the seasons of the year. Overall, the lowest percentage (%) of detection was recorded in spring while the highest was recorded in autumn (%). For Bloukrans River samples, the highest prevalence (80%) was recorded in autumn, compared to summer (60 %), spring (60 %), and winter (60 %) samples (Fig. 4). For Swartkops River, *Campylobacter* was detected in 67% of the winter and autumn samples, while spring and summer were recorded in 50% of samples (Fig. 5). However, based on the outcome of the logistic regression analysis performed, the sampling season had no significant effect on the detection of *Campylobacter* for the Bloukrans River ($\chi2 = 0.66$, $p = 0.883$) and the Swartkops River ($\chi2 = 0.69$, $p = 0.877$).

4. Discussion

This study investigated the spatial and temporal distribution of *Campylobacter* spp. and selected antibiotic-resistant genes in river water. The influence of meteorological conditions, physicochemical parameters, season of the year, and sampling site on the detection of *Campylobacter* (dependable variable) were assessed. This ascertained whether these rivers are potential sources of *Campylobacter* and the periods of greatest *Campylobacter* exposure from the rivers to humans. Physicochemical variables of the river water is first considered, as it influences the occurrence of *Campylobacter* in the environment [7].

The results obtained in this study suggest that the physiochemical properties (pH, dissolved oxygen level, turbidity, and electrical conductivity) of the river water are conducive to *Campylobacter* survival. It is notable that at the prevalence of *Campylobacter* was high at sites dissolved oxygen was low and electrical conductivity and turbidity were high. The findings from this study correlate with those from previous studies. High turbidity, high electrical conductivity, and low dissolved oxygen favor the survival of *Campylobacter* in river water [27–29,30]. Turbidity may result from the high concentration of dissolved particles, and provide nutrients, which thereby favours the survival of bacteria, including *Campylobacter* that are present in the river [31]. In previous studies, a strong positive correlation has been found between turbidity and *Campylobacter* presence in river water [32,33]. Low dissolved oxygen at sites with a higher prevalence of *Campylobacter* is also expected because their survival is better at low dissolved oxygen concentration [34,35]. Nevertheless, the physicochemical characteristics of the river water did not show a significant influence on the occurrence of *Campylobacter* in both the Bloukrans and Swartkops rivers.

It is noteworthy that the water temperatures (>16 °C), which are measurable for Bloukrans and Swartkops rivers in this study, may not be suitable for the bacteria to grow. It has been suggested that the warm climate causes higher temperatures and longer UV exposure periods, which can lead to lower potential survival and consequent isolation rates [36]. However, one report has shown that *Campylobacter* can survive at these temperatures [37]. Besides, these bacteria have acquired the ability to harsh environmental conditions. Moreover, *Campylobacter* species exhibit different survival patterns, ascribed to their high genetic diversity and therefore interspecies variations in genes for responding to stress are observed [38]. For example, *Campylobacter jejuni* can survive longer in the viable but non-culturable (VBNC). In VBNC form in freshwater, *Campylobacter jejuni* retains infectivity [12]. Furthermore, *Campylobacter jejuni* is also more tolerant to stress conditions compared to *Campylobacter coli* [12,39]. Therefore, may be more likely to be detected in the rivers.

This study reports a prevalence of 66.7% for the Bloukrans River and 55.33% for the Swartkops River. The prevalence of *Campylobacter* spp. in river systems can vary by region and country as reported in previous studies. For instance, *Campylobacter* occurrence has been reported to be 25% in Australia [40], 26 % in Canada [41], 35.7% in Ghana [42], 41.5% in India [43] 46.6%–53.3% in France [44], 53.3% in Norway [45] and 91% in the Yarra River estuary, Australia [46]. *Campylobacter* occurrence (66.67 %) reported in the Bloukrans River in this study is similar to the 68.7% reported in Nigeria [47] and 33–63% in Canada [48]. The differences observed in the prevalence from the different studies could also be a result of different methods used for detection, local anthropogenic activities, and the physicochemical characteristics of the river water [49].

The sampling site had a significant effect on the detection of *Campylobacter* for both rivers. Variations in the occurrence of *Campylobacter* at the different sites on both rivers, which is observed, reflects the various level and types of anthropogenic activities that occur, respectively. High prevalence of *Campylobacter* was observed for sites influenced by wastewater, agriculture, human settlements and solid waste. This high prevalence of *Campylobacter* at sites impacted by anthropogenic activities correlates with what has been reported in literature [13,50–52]. *Campylobacter* was detected at sites which are used for livestock watering, indicating that animals can act as the source of this pathogen (through faecal matter) and also the transmission route (from river water to humans).

Table 3
Mean meteorological conditions (standard deviation) for the sampling days in each season.

Season	Rainfall (mm)		Humidity (%)		Air temperature (°C)		Atmospheric pressure (kPa)	
	BR	SR	BR	SR	BR	SR	BR	SR
Winter	0 (0)	101.35 (0.35)	41.8 (9.31)	62.67 (9.81)	20.6 (3.29)	21 (1.55)	141.54 (31.55)	0.07 (0.1)
Spring	0 (0)	100.73 (0.19)	48.8 (23)	64 (2.19)	19.8 (4.38)	21 (1.1)	165.28 (77.89)	0.2 (0.31)
Summer	0.12 (0.27)	100.9 (0)	46.4 (13.15)	57 (6.2)	27.2 (1.1)	26.67 (1.03)	157.12 (44.48)	0.13 (0.21)
Autumn	0 (0)	101.07 (0.19)	36.6 (8.76)	60.5 (9.31)	26.8 (1.1)	23 (2.19)	123.92 (29.69)	0.55 (0.05)

BR= Bloukrans River, SR= Swartkops River.

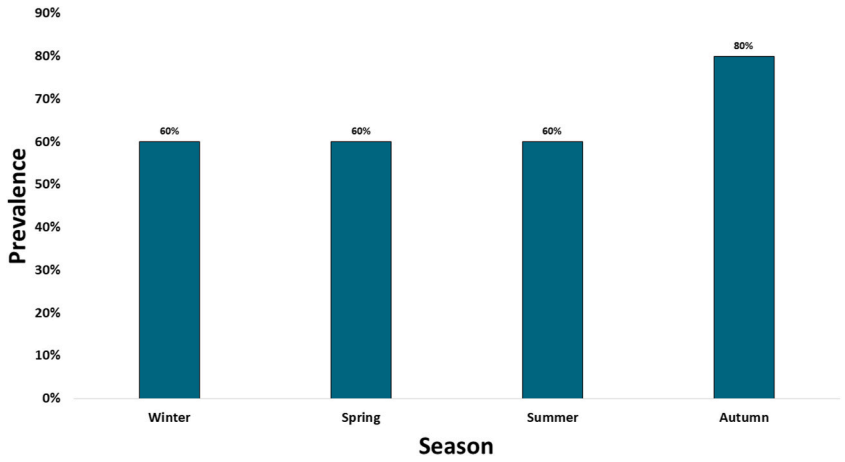

Fig. 4. Seasonal occurrence of *Campylobacter* species for the Bloukrans River, Eastern Cape South Africa.

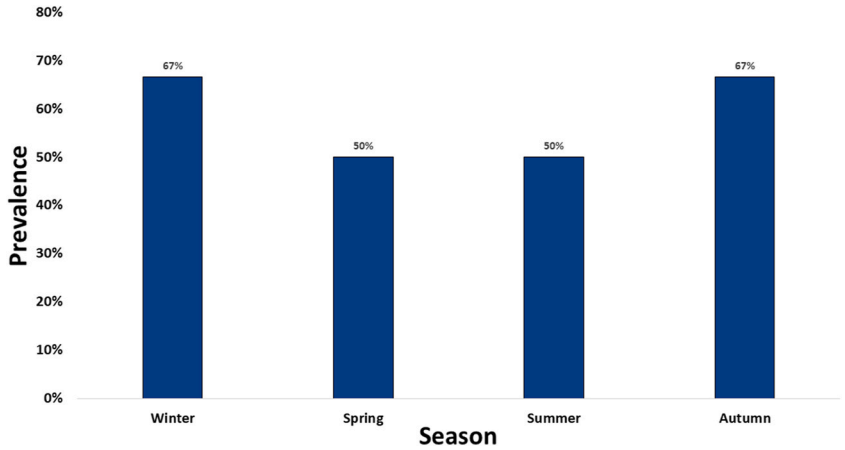

Fig. 5. Seasonal occurrence of *Campylobacter* species for the Swartkops River, Eastern Cape South Africa.

The occurrence of *Campylobacter* in rivers often suggests recent faecal contamination by domestic animal, runoffs from farm animal manure, birds, and insufficiently treated wastewater or leakages from nearby septic tanks [49].

Meteorological conditions of the sampling day had no significant effect on the detection of *Campylobacter* for both rivers. There is limited information on the influence of meteorological conditions on the survival of *Campylobacter* in river water. The few studies available, suggest that meteorological conditions of the sampling day affect the detection of *Campylobacter* in rivers. According to the studies, low air temperatures, 14–15 °C or air temperature below 18 °C support the occurrence of *Campylobacter* spp. in rivers [53,54]. In addition, *Campylobacter* detection is reported to be positively associated with an increase in rainfall [1].

This study did not show any significant effect of sampling season on the occurrence of *Campylobacter* for both rivers. However, the higher occurrence of *Campylobacter* in autumn for the Bloukrans River, and in summer for the Swartkops River is notable. Similarly, a high occurrence of *Campylobacter jejuni* and *Campylobacter coli* in river water was observed in autumn [54]. Another study reported seasonal variation in levels of *Campylobacter* contamination in rivers and high levels were recorded in summer [7]. Similar to the results obtained in this study, there was no significant seasonal effect on *Campylobacter* isolation in river water used for domestic consumption in Brittany, France [44]. However, season and temperature had significant effects on the occurrence of *Campylobacter* in the study by Wilkes et al. [53].

Furthermore, the *Campylobacter jejuni* detected in the river water in this study express antibiotic-resistant genes. The *Campylobacter tetO* gene detected in the positive water samples is plasmid-borne and is acquired by *Campylobacter* through horizontal gene transfer [55,56]. The gene gives rise to increase in resistance to tetracycline. The binding of the *tetO* genes to an open A site induces a change in the conformational leading to the release of the attached tetracycline molecule and so protein elongation is not interrupted [57]. The presence of *tetO* in the water samples is expected given the high rate of resistance against tetracycline observed among *Campylobacter* isolates from humans and the use of this antibiotic in veterinary medicine. Furthermore, the detection of multidrug efflux pump c*me*ABC genes in the rivers is a serious concern. The c*me*ABC genes encode proteins of different structures involved in extruding antimicrobials [58]. A study in South Africa also reported the detection of *Campylobacter jejuni* isolates that exhibited multidrug

resistance in estuarine water samples [51].

The increase in antibiotic-resistant *Campylobacter* in these rivers is being driven by anthropogenic activities such as poor solid waste disposal, runoff from human settlements, livestock grazing, and discharge of poorly or raw wastewater. Additionally, the livestock industry in South Africa heavily relies on use of antibiotics as prophylaxis or growth promoters. This has resulted in high and inappropriate use of antibiotics especially in intensive livestock production. This is worsened by weaknesses in the guidelines for veterinary antibiotic use in South Africa [59,60]. The guidelines for veterinary antibiotic use permit the use of erythromycin, ampicillin, streptomycin, and tetracycline in livestock [59,61,62]. In South Africa, antibiotics for agricultural usage are less restricted compared to most developed countries. For instance the Fertilizers, Farm Feeds, Agricultural Remedies, and Stock Remedies Act 36 of 1947 allows the non-prescription sale of antibiotics registered under stock feeds. This low restriction on antibiotic use leads to the high usage of antibiotics by farmers in South Africa [51,63].

A major limitation of this study is that the abundance and densities of *Campylobacter* spp. and their genes were not quantified in the samples, and this is a critical requirement to effectively determine the associated human health. More research is needed to quantify *Campylobacter* in rivers and link human *Campylobacter* infections to specific sources in rivers.

5. Conclusion

This study highlights a widespread and continual occurrence of *Campylobacter* spp. in the Bloukrans and Swartkops rivers, which are potentially antibiotic-resistant. The occurrence of *Campylobacter* in water is linked to the physical condition of the rivers and anthropogenic activities prevailing in the catchment. *Campylobacter* infection may be endemic in South Africa, and the Bloukrans and Swartkops rivers are potential sources of *Campylobacter* exposure to humans. The information on the factors promoting the survival of *Campylobacter,* provided in this study is useful in monitoring *Campylobacter* in rivers. Water from these rivers is utilised for irrigation of crops (mainly vegetables), spiritual/cultural activities, recreation (mainly for children), drinking water for animals, and to a minimal extent fishing. These rivers are an interface for human and animal activities and are a risk factor for the spread of *Campylobacter* species. It is therefore critical that the management of urban rivers is improved to prevent microbial contamination. Transmission of pathogens through contact with river water may play a role in the epidemiology of enteric diseases, including Campylobacteriosis in this region. This study contributes valued insights into the environmental factors influencing *Campylobacter* dynamics, informing steered strategies for water quality management and public health interventions.

Data availability

The data used and/or analysed during this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Mary Chibwe: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Oghenekaro Nelson Odume:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition. **Chika Felicitas Nnadozie:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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